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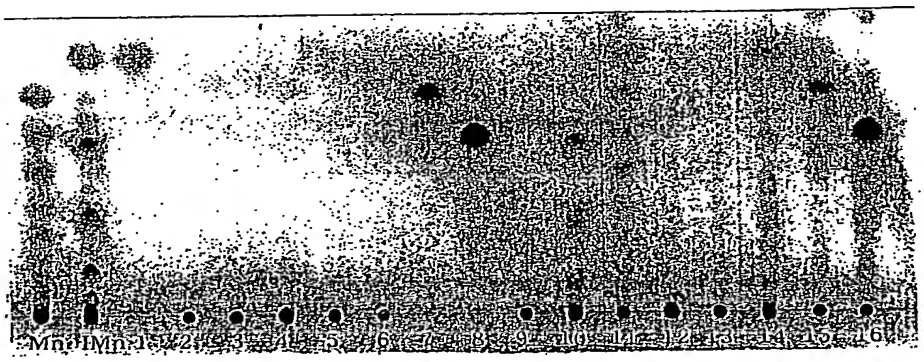


(57) Abstract: The present invention relates to a enzyme of repressing plaque formation and degrading plaque, a DNA sequence encoding the enzyme, the expressing host cell, methods for producing said enzyme, and the isolated and purified enzyme. The invention also relates to compositions comprising the enzyme.

ENZYME WITH THE REMOVAL

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention relates to an enzyme which can effectively repress formation of plaque or degrade the plaque, gene for encoding the enzyme, and a process for preparing the enzyme and the expressed cell. More specifically, the invention relates to an enzyme which can effectively repress formation of plaque produced from *Lipomyces starkeyi* KSM 22 mutant and degrade the plaque, gene for encoding the enzyme, a process for producing said enzyme and the expressed cells, and a composition comprising the enzyme.

Description of the Prior Art

Generally, film formed on surface of a tooth (plaque) consists of intimately packed bacteria and non-cellular substances. The main polysaccharide component of plaque is water-insoluble glucan or mutan, which occupies about 20% of total dry weight of plaque, is one of the essential cause of tooth-decay. By way of investigations of the structure of glucan produced by *Streptococcus mutans*, it has been reported that insoluble glucan essentially comprises α-1,3-, α-1,4- and α-1,6-D-glucocide bonds. Thus, the activity of degrading mutan and that of decomposing starch and dextran are required in order to effectively remove plaque.

Conventionally, repression of growth of *Streptococcus mutans* (hereinafter, abbreviated to as "*S. mutans*") has been suggested as a means to repressing plaque

formation or tooth-decay, so that an antibiotic substance to repress growth of S. mutans or a fluorine compound was contained in a product for oral use such as mouth washer. Fluorine, as a representative compound of anti-cavity substance, has severe adverse effect of white spot on tooth enamel even at a very low concentration as well as intense toxicity and air pollution, even though it has an effect of repressing growth of bacterial source of tooth-decay. Enzyme such as dextranase has been also used for repressing tooth-decay, but the activity has not been clearly demonstrated yet.

U.S. Pat. 5,741,773 provided a toothpaste composition comprising glycomacropeptide having anti-plaque and activity preventing tooth-decay. Such an art, however, simply relates to repression of growth of the bacterial source of tooth-decay, but could not disclose repression of plaque formation or degradation of already formed plaque.

Investigations to employ an enzyme DEXAMase which decomposes polysaccharides of various structures in degradation of already formed plaque have been made by the present inventors. Patent applications regarding a microorganism (*Lipomyces starkeyi* KFCC-11077) producing an enzyme for degrading various polysaccharides, the enzyme and a composition comprising the same are pending in Korea and in the United States.

SUMMARY OF THE INVENTION

The present invention is contrived by said requirements to solve the problems described above.

Accordingly, the present invention has an object to provide novel enzyme having activity to repress formation of plaque or to degrade plaque, and gene encoding the enzyme.

Another object of the present invention is to provide a cell expressing said

gene.

Still another object of the invention is to provide a process for preparing said enzyme and gene.

Still further object of the invention is to provide a commercially useful composition comprised of said enzyme.

In order to achieve the objects, the present invention provide protein comprising amino acid residue of sequence information 1 or mutants thereof, and enzyme that degrades insoluble glucan having both dextranase activity and amylase activity as a part thereof.

Further, the present invention provides said protein or its mutants, gene or mutants of sequence information 2 encoding a part thereof, and/or the segments. Preferably, said mutants of protein comprises not less than 80% of homology with amino acid sequence of sequence information 1, and said gene mutants comprises not less than 80% of homology with gene base sequence of sequence information 2.

The present invention also provides transformed cells which express said gene. The cells are preferably prokaryotic cell or eukaryotic cell. The most preferable one is E. coli KCTC 10024BP.

The present invention further provides a process preparing a purified enzyme which has both dextranase activity and amylase activity and degrades insoluble glucan, comprising the steps of culturing said cell, expressing an enzyme from the cultured cell, and purifying the expressed enzyme; an enzyme prepared by said process; and a composition comprised of the enzyme.

BRIEF DESCRIPTION OF THE DRAWINGS

The present invention will be explained in terms of exemplary embodiments described in detail with reference to the accompanying drawings, which are given only by way of illustration and thus are not limitative of the

present invention:

Fig. 1 shows the effect of pH on activity and stability of DEXAMase: (a) shows the effect on the activity, while (b) shows the effect on the stability.

Fig. 2 shows the effect of temperature on the stability of DEXAMase.

Fig. 3 shows a TLC photograph of polysaccharides hydrolyzed by using DEXAMase prepared from recombinant clone pBDEXAM. In the photograph, Mn and IMn shows a series of maltodextrin, lane 1 shows dructose, lane 2 shows dextran, lane 3 shows starch, lane 4 shows inulin, lane 5 shows levan, lane 6 shows mutan, lane 7 shows isomaltose, lane 8 shows maltose, lane 9 shows cellular supernatant, lane 10 shows the TLC photograph after reaction with dextran, lane 11 shows one after reaction with starch, lane 12 shows one after reaction with inulin, lane 13 shows one after reaction with levan, lane 14 shows one after reaction with mutan, lane 15 shows one is after reaction with isomaltose, and lane 16 shows one after reaction with maltose.

Fig. 4 shows bonding of DEXAMase having the activity of dextranase and amylase on hydroxyapatite.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Now, the present invention is described in more detail.

In order to obtain DEXAMase gene, *L. starkeyi* KSM 22 was cultured in a starch-containing medium, poly(A) + RNA were obtained, cDNA were prepared by the poly(A) + RNA and the cDNA was ligated to Uni-ZAP XR vector. The phage lysate was transformed to pBluescript phagemid via segmenting *in vivo* after ligation. Then, transformation was subjected thereto by using *E. coli* XL1-Blue to prepare cDNA library. The cDNA library gave clone pBDEXAM formed apparent halo due to decomposition of dextran in a medium containing blue dextran and IPTG.

It has been reported that *Lipomyces starkeyi* (hereinafter, abbreviated to as "*L. starkeyi*") produces endodextranase (EC 3.2.1.11) that degrades dextran and α-amylase that degrades starch. The microorganism is applied in the field of food, but it has not been reported that the microorganism produces antibiotic material or other toxic material.

It has been reported that dextranases produced by microorganism are generally inducible enzymes except some bacterial dextranases. The present inventors have disclosed *L. starkeyi* ATCC 74054 which always produces both dextranase and amylase (U.S. Pat. 5,229,277) with characterizing the properties of the enzyme prepared by the microorganism that produces dextran of small size by using sucrose and starch. The present inventors also has a patent application pending that relates to a microorganism producing dexamase enzyme which can degrade dextran and starch at the same time by using *Lipomyces starkeyi* KFCC-11077, an enzyme of the microorganism, and a composition comprised of the enzyme.

The enzyme prepared from the gene (dexam) of the present invention degrades not only dextran and starch but also insoluble glucan. Hereinafter, the enzyme according to the present invention is referred to as "DEXAM enzyme".

Dexamase of the present invention, in case that dextran is used as the substrate, essentially produces glucose, isomaltose and branched tetrose, and small amount of branched pentose and branched hexose. When starch is used as the substrate, the enzyme essentially produces glucose, maltose, maltotriose and maltotetrose, and various maltooligosaccharides as well.

Since DEXAM enzyme of the present invention can degrade levan (polymer of β-fructan), it can effectively degrade fructan, one of the component of plaque formation.

Thus, DEXAM enzyme of the present invention effectively degrades both

soluble glucan and insoluble glucan. In addition, the enzyme can repress formation of plaque or remove the plaque already formed by inhibiting aggregation of glucan and bacteria, thereby showing activity on preventing tooth decay.

From the experiments by using hydroxyapatite, which consists of similar components as tooth, DEXAM enzyme showed the property of retaining the bond to hydroxyapatite, being suggested that DEXAMase can be retained on tooth.

Further, the present invention relates to novel microorganism comprising gene encoding DEXAMase. The *E. coli* strain of the present invention was deposited in KCTC at Yuseong-gu, Daejeon on July 26, 2001 with Deposit No. KCTC 10024BP.

The present invention also relates to a process for preparing DEXAM enzyme. The process comprises the steps of culturing pBDEXAM clone and isolating DEXAM enzyme from the culture medium. As pBDEXAM can produce DEXAMase by using not only expensive dextran but also glucose, fructose, sucrose or starch, the process is economically advantageous. The properties of DEXAM enzyme obtained from pBDEXAM is substantially identical to those of DEXAMase isolated from *L. starkeyi* KFCC-11077.

In addition, the present invention relates to a composition for preventing tooth-decay that comprises DEXAM enzyme. The activity of the enzyme of the present invention can be retained in commercially available mouth washing solutions for a long time, with showing intense tolerance against enzyme inhibitors. Thus, the composition containing the enzyme of the present invention can be employed in various oral protective products such as toothpaste and mouth washing solution. The composition comprising the enzyme of the present invention can be applied to food such as chewing gum, soft drink or dairy products. The specific constituents of the composition may be appropriately determined by a person having ordinary skill in the technical field without difficulty.

Now, the present invention is described in more detail by referring to Examples which are not limitative:

In the present invention, the strain employed as DNA donor for preparing cDNA library was *Lipomyces starkeyi* KFCC 11077, which constitutively produces DEXAMase having dextranase and amylase activity. *Escherichia coli* XL1-Blue and λ-phage Uni-ZAP XR (Stratagene, USA) were employed as host cells and plasmid [phagemid] for preparing cDNA library.

L. starkeyi KFCC 11077 was cultured in LMS medium under aerobic condition at 28°C. The LMS medium comprised 1 %(w/v) of starch, 1%(v/v) of mineral solution and 0.3%(w/v) of yeast extract. Composition of the mineral solution was 2%(w/v) of $MgSO_4 \cdot 7H_2O$, 0.1%(w/v) of NaCl, 0.1%(w/v) of $FeSO_4 \cdot 7H_2O$, 0.1%(w/v) of $MnSO_4 \cdot H_2O$, and 0.13%(w/v) of $CaCl_2 \cdot 2H_2O$. As the medium for bacterial growth, LB (1% of tripton, 0.5% of yeast extract, 1% of NaCl, pH 7.3) and LBA (LB containing 50 μg of ampicillin/ml) were employed.

Example 1: Isolation of poly(A) + RNA from *L. starkeyi*

In LMS liquid medium, L. starkeyi KFCC-11077 was inoculated and cultured at 28°C for 36 hours. At the middle phase of exponential growth, the culture solution was centrifuged (6,500 xg) to obtain a cell pellet. The cell pellet was dissolved in a mixture of GIT solution [4M guanidine isothiocyanate, 25 mM sodium citrate (pH 7.0)], 0.5% lauroylsarcosyl dissolved in distilled water treated with 0.1% DEPC and 0.1 M 2-mercaptoethanol. Glass bead washed with acid and equal amount of phenol (pH 4.0) were added thereto and the mixture was subjected to vortex for 2 minutes. To the supernatant solution obtained after centrifugation, isopropanol was added to precipitate total RNA. From the oligotex-mRNA complex formed, mRNA was isolated by the use of oligotex resin. (Oligotex mRNA kit, Qigen).

Example 2: Preparation and screening of *L. starkeyi* cDNA

By the use of ZAP-cDNA Synthetic Kit (Stratagene, U.S.A.), cDNA was prepared from poly(A) + RNA (5 μg) obtained by culturing in a medium containing starch for 36 hours. The prepared cDNA was isolated as the size of not less than 500 bp by spin column separation. Then it was ligated with Uni-ZAP XR vector that had been treated with EcoRI-Xho. The ligated phage cDNA was subjected to packaging in vitro by using Gigapack Gold Kit (Stratagene, U.S.A.). The phage lysate thus obtained was transfered to pBluescript via excision in vivo, transformed with *E. coli* XL1-Blue, and cultured in LBA solid medium overnight. When recombinant colonies were grown up, top agar comprising 0.8% of agarose, 0.2% of blue dextran and 4 μl of 0.1M IPTG was placed on the plate, which was cultured at 28°C for 1-2 days to select the colonies in which blue dextran is hydrolysed to form obvious halo.

The selected colonies are named as pBDEXAM.

Example 3: Assay of base and amino acid sequence of cDNA clones

Plasmid DNA for assay of base sequence was prepared according to alkalinelysis method. The assay of base sequence was performed by means of GeneAmp 9600 thermal cylinder DNA sequencing system (model 373-18, Applied Biosystems, U.S.A.) and ABI PRISM Cycle Sequencing Kit (Perkin Elmer Corp. U.S.A.).

The base sequence of DNA fragment (5.4 kb) having glucanhydrolase gene had one open reading frame (ORF) comprised of 2679 bp of bases. Presumed amino acid sequence locates from the starting codon (ATG) at the 874th nucleotide position of the identified base sequence to the terminating codon (TGA) at the 3552nd position. The structural gene consists of 892 amino acids, and calculated molecular weight is 99.1 kDa.

Example 4: Selection of strain which expresses DEXAM gene

In order to confirm activity in the supernatant, clones which showed activity on the plate was subjected to isopropyl-α-D-thiogalactosidase (IPTG) induction. The selected cononies were cultured in 5 ml of LBA liquid medium at 37°C overnight. One percent (1%) (v/v) thereof was inoculated in LB medium again and cultured until it was 0.5 at OD_{600nm}, and IPTG was added to make the final concentration 0.1 mM. After culturing at 25°C overnight, the culture solution was centrifuged (6,500 xg, 15 minutes) to recover supernatant. After mixing the supernatant with 1% of dextranase or 1% of starch by 1:1; the mixture was reacted for 20 hours to confirm the activity.

Example 5: Preparation of coenzyme solution

Clone pBDEXAM was cultured in LBA liquid medium at 37°C overnight, of which 1% was inoculated again in LB medium to be cultured to make 0.5 at OD_{600nm}. IPTG was added until the final concentration became 0.1 mM, and the mixture was cultured at 25°C overnight. The culture solution was centrifuged (6500 xg, 15 minutes) and the supernatant solution was obtained to be used as the first enzyme solution. In order to concentrate the recovered first enzyme solution, enzyme not less than 30 kDa was concentrated by means of 30K cut-off hallow fiber (Milipore, U.S.A.). In order to remove the components of culture medium, it was concentrated by six times by exchanging four times with 20 mM citrate/phosphate buffer (pH 5.5) [2 L each time]. To the concentrate, polyethyeneglycol (PEG, MW=150,000 – 20,000) was added, and the mixture was reacted at 4 °C to concentrate by 20 times further. The PEG concentrate was dialyzed to 20 mM citrate/phosphate buffer (pH 5.5), and the dialyzed solution was used as the coenzyme solution for measuring protein activity.

Example 6 : Measurement of enzyme activity

In order to measure reducing value, DNS (3,5-dinitrosalicylic acid) method was employed. DNS reagent (100 μl) and enzyme reaction mixture (50 μl) and distilled water (50 μl) were added, and the mixture was reacted at 80°C for 10 minutes, and chilled for about 15 minutes. Then, absorbance was measured at 550 nm. Dextranase activity of DEXAM enzyme was determined by measuring the amount of isomaltose produced after adding enzyme solution to buffer containing 2% dextran and reacting the mixture at 37°C for 15 minutes. One unit of dextranase is defined by the amount of enzyme that produces 1 μM of isomaltose after reaction at 37°C for 1 minute by using dextran as substrate. Amylase activity is defined by the amount of enzyme that produces 1μM of maltose after adding the enzyme to buffer containing 2% of starch and reacting the mixture at 37 °C.

The enzyme activity of dextranase and amylase of the coenzyme solution (DEXAM) obtained from recombinant clone pBDEXAM was examined. As a result, it was confirmed that dextranase activity was 0.16 unit/ml and amylase activity was 0.25 unit/ml.

Example 7: Optimal activity and stability of DEXAM enzyme depending on pH or temperature

Optimal pH of dextranase and amylase activity of DEXAM enzyme was determined by reaction rate in the pH range of 4 – 9 (by interval of pH 1.0). For the experiment, 20 mM citrate-phosphase buffer (pH 4.0), citrate/phosphate buffer (pH 5-6) and sodium phosphate buffer (pH 7-9) were employed, and dextranase and amylase activity was determined by using DNS (3,5-dinitrosalicylic acid) method after reaction at 37°C for 48 hours. The pH stability of enzyme was measured after adding enzyme to each buffer and standing the mixture at 22°C for

48 hours.

Optimal temperature of enzyme activity was determined by measuring reaction rate after adding the enzyme at various temperatures (20-90°C, 10°C interval) and standing the mixture for 30 minutes. The temperature stability was obtained by measuring residual activity after placing the enzyme at various temperatures for 30 minutes.

Dextranase and amylase of DEXAMase showed optimal activity at pH 5.5. Amylase activity retained not less than 80% of optimal activity in the range of pH 5.5 – 7, while dextranase activity did in the range of pH 4.0 – 9.0 (Fig. 1, Table 2).

The temperature of maximum enzyme activity of dextranase and amylase was 40°C and 50°C, respectively. The dextranase activity and amylase activity retained 80% of initial activity at 50°C or lower temperature and 60°C or lower, respectively. (Fig. 2 and Table 3)

Example 8: Degrading properties of DEXAM enzyme on various substrate

Degradation of various substrate by the enzyme solution was examined. In addition to dextran, water-soluble starch and glucan, 1% (w/v) aqueous solutions of macromolecular polymers having various structures were prepared, and the mixture was mixed with enzyme concentrate in an equivalent amount to be reacted at 37°C for 5 days. The hydrolyzed product thus obtained was analyzed. For the conformation of hydrolysis of macromolecular polymers, levan (polysaccharide wherein D-fructose is bonded in the position of β-2,6), inulin (polysaccharide wherein D-fructose is bonded in the position of β-2,1) or mutan (polysaccharide wherein D-glucose is bonded in the position of α-1,3), or maltose (4-D-α-D-glucopyranosyl-D-glucose; disaccharide wherein two moleculues of D-glucose are bonded in the position of α-1,4) or isomaltose (6-D-α-D-

glucopyranosyl-D-glucose; disaccharide wherein two molecules of D-glucose are bonded in the position of α-1,6) was employed.

Via reaction with dextran substrate, 14.0% of glucose, 31.2% of isomaltose and 33.5% of isomatotriose were essentially produced. Branched oligosaccharides were produced as well. Via reaction with starch, glucose, maltose, isomaltotiose and maltotetrase were essentially produced in the amount of 28.2%, 18.4%, 24.7% and 28.6%, respectively. Thus, DEXAM is considered that it performs endo-dextranase and endo-amylase type reaction in the reaction with dextran and starch (Fig. 3).

In order to examine degradation property of DEXAM enzyme produced from clone pBDEXAM, macromolecular polymers having various structures are reacted with the enzyme. As a result, it showed hydrolytic properties on levan and inulin having β-bond as well as macromolecular polymers containing α-1,3-D-glucoside bond such as mutan. In addition, disaccharides such as maltose and isomaltose were also hydrolyzed (Fig. 3).

Example 9: DEXAMase bond to hydroxyapatite (HA)

Having ability to directly attach to bones, calcium phosphate ceramics are widely used as alternative material of bones. Among them, hydroxyapatite (HA), which has a lot of identical crystallographic properties to natural apatite existing in bones, is widely used as alternative material in research of artificial bones and teeth. Coherence of DEXAMase to HA was examined. Hydroxyapatite (Bio-Gel HTP, Bio-Rad Laboratories, Richmond CA) was suspended in 10 mM phosphate buffer (pH 6.8). DEXAMase was dissolved in the same buffer solution. The enzyme activity was 10 U/ml. Each 200 μl of HA and enzyme were mixed, and absorption was performed for 60 minutes. After washing unabsorbed enzyme, the adsorbed enzyme was gradually eluted by using 10, 50, 100, 200, 300,

400, 500 mM of phosphate buffer (pH 6.8) containing 1 M NaCl. Eluted enzyme fractions are collected, and dextranase and amylase activity was measured.

As can be shown in Fig. 4, dextranase and amylase of DEXAMase were eluted in 200 – 300 mM of hydroxyapatite. From the result, it is confirmed that DEXAMase has strong cohesion to hydroxyapatite, so that it remains on teeth for a long time.

Example 10: Effect of inhibitors on dextranase and amylase activity

Ethanol (5%) did not affect on activity of each enzyme, and sodium fluoride did not affect on amylase. However, EDTA reduced about 40% of initial activity of both enzymes, and 0.05% and 0.5% SDS reduced about 35~40% of the activity, while 10~20% ethanol reduced about 45% of dextranase activity and 15-30% of amylase activity (Table 4).

Example 11: Stability of dextranase and amylase against mouth washing components

Sodium benzoate gave no affect on dextranase and amylase activity of DEXAMase. In case of 0.01% of chlorhexidine, 90% of the activity was retained. However, in case of cetylpyridinium chloride, enzyme inactivity appeared depending on the concentration. When the concentration was 0.01%, about 90% or more activity was retained, while about 60% and 48% of dextranase and amylase activity was reduced, respectively, in case of 1% concentration (Table 5).

[Table 1] DEXAMase activity of pBDEXAM clone

Substrate	Before reaction	After reaction	OD_{550}	Glucose (μg)	Enzyme activity (unit[a]/ml)
Dextran	0.046	0.089	0.043	13.21989	0.163209
Starch	0.042	0.205	0.163	20.11842	0.248376

In the Table, one unit[a] of dextranase or alylase is defined as the amount of enzyme that can produce 1 μmole of glucolse in an hour by using 2% (w/v) of dextran or water-soluble starch.

[Table 2] Effect of pH on DEXAMase activity and stability

	Dextranase	Amylase
Optimum pH	5.5	5.5
Stable pH range	4.0 – 9.0	5.5 – 7.0

In the Table, stable pH range means that residual activity is not less than 60% of the initial activity.

[Table 3] Effect of temperature on stability of DEXAMase

	Dextranase	Amylase
Stable temperature range (°C)	≤ 50	≤ 60

In the Table, stable temperature range means that residual activity is not less than 80% of the initial activity.

[Table 4] Dextranase activity and amylase activity of DEXAMase on various compounds

Inhibitor	Concentration	Relative activity (%)	
		Dextranase	Amylase
Control		100	100
EDTA	10 mM	68	60
SDS	0.05 %	62	75
	0.5 %	60	61
Fluorine	0.05 %	86	98
Ethanol	1 %	89	100
	5 %	100	100
	10 %	44	82
	20 %	46	71

[Table 5] Dextranase activity and amylase activity of pBDEXAM on chemical components used in mouth washing products

Chemical component	Concentration	Relative activity (%)	
		Dextranase	Amylase
Control		100	100
Sodium benzoate	1 %	99	100
Chlorhexidine	0.01 %	98	100
	0.02 %	71	100
	0.05 %	68	97
	0.1 %	83	94
Cetylpyridinium chloride	0.001 %	100	100
	0.01 %	98	92
	0.05 %	72	85
	0.1 %	54	71
	0.5 %	39	70
	1 %	40	52

As can be shown from the above constitution of the invention, DEXAMase produced by *Lipomyces starkeyi* KSM 22 according to the present invention is a single protein of about 100 kDa size, and shows both dextranase (EC 3.2.1.11) activity and amylase (EC 3.2.1.1) activity. As a result of assay of DNA base sequence, the base sequence of DNA segment (5.4 kb) had one open reading frame (ORF) comprised of 2679 bp of bases. The gene of presumed structure consists of 892 amino acids, and the molecular weight was about 99.1 kDa.

As a result of confirming dextranase and amylase activity of coenzyme, dextranase activity was 0.16 unit/ml, while amylase activity was 0.25 unit/ml. Final reaction products of dextranase and amylase with dextran and starch were typical products of endo-dextranase and endo-amylase. Dextranase activity essentially produced glucose (14.0%), isomaltose (31.2%) and branched tetrasaccharide (33.5%) as well as branched pentasaccharide. Amylase activity essentially produced glucose (28.2%), maltose (18.4%), matotriose (24.7%) and

maltotetrase (28.6%). In order to examine properties of DEXAMase to degrade various carbohydrates, the enzyme was reacted with macromolecular polymers of various structures. As a result, it showed the property of hydrolyzing macromolecular polymer comprising α-1,3-D-glucoside bond such as mutan as well as fructan comprising β-bond such as levan and inulin.

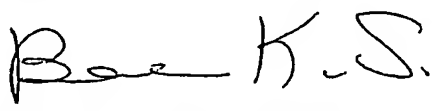
BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSE OF PATENT PROCEDURE

INTERNATIONAL FORM

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT

issued pursuant to Rule 7.1

TO : Lifenza Co., Ltd.
2nd Floor Samwoo Bldg.,
#732-24, Yeoksam-dong, Kangnam-gu, Seoul 135-514,
Republic of Korea

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR: *Escherichia coli* **XL1-Blue/pBDEXAM**	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: **KCTC 10024BP**
II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION	
The microorganism identified under I above was accompanied by: [x] a scientific description [ ] a proposed taxonomic designation (Mark with a cross where applicable)	
III. RECEIPT AND ACCEPTANCE	
This International Depositary Authority accepts the microorganism identified under I above, which was received by it on **July 26 2001.**	
IV. RECEIPT OF REQUEST FOR CONVERSION	
The microorganism identified under I above was received by this International Depositary Authority on and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on	
V. INTERNATIONAL DEPOSITARY AUTHORITY	
Name: Korean Collection for Type Cultures Address: Korea Research Institute of Bioscience and Biotechnology (KRIBB) #52, Oun-dong, Yusong-ku, Taejon 305-333, Republic of Korea	Signature(s) of person(s) having the power to represent the International Depositary Authority of authorized official(s):  BAE, Kyung Sook, Director Date: **July 30 2001**

Form BP/4 (KCTC Form 17) sole page

WHAT IS CLAIMED IS:

1. An enzyme comprised of amino acid residue of sequence information 1, its mutant or a part thereof, which degrades insoluble glucan and has both dextranase activity and amylase activity.

2. Gene, mutant and/or segment of sequence information 2 which encodes the protein, its mutant or a part thereof claim 1.

3. The protein according to claim 1, wherein the mutant has not less than 80% of homology with the amino acid sequence of sequence information 1.

4. The DNA according to claim 1, wherein the mutant has not less than 80% of homology with the gene base sequence of sequence information 2.

5. The transformed cell which express DNA of claim 2.

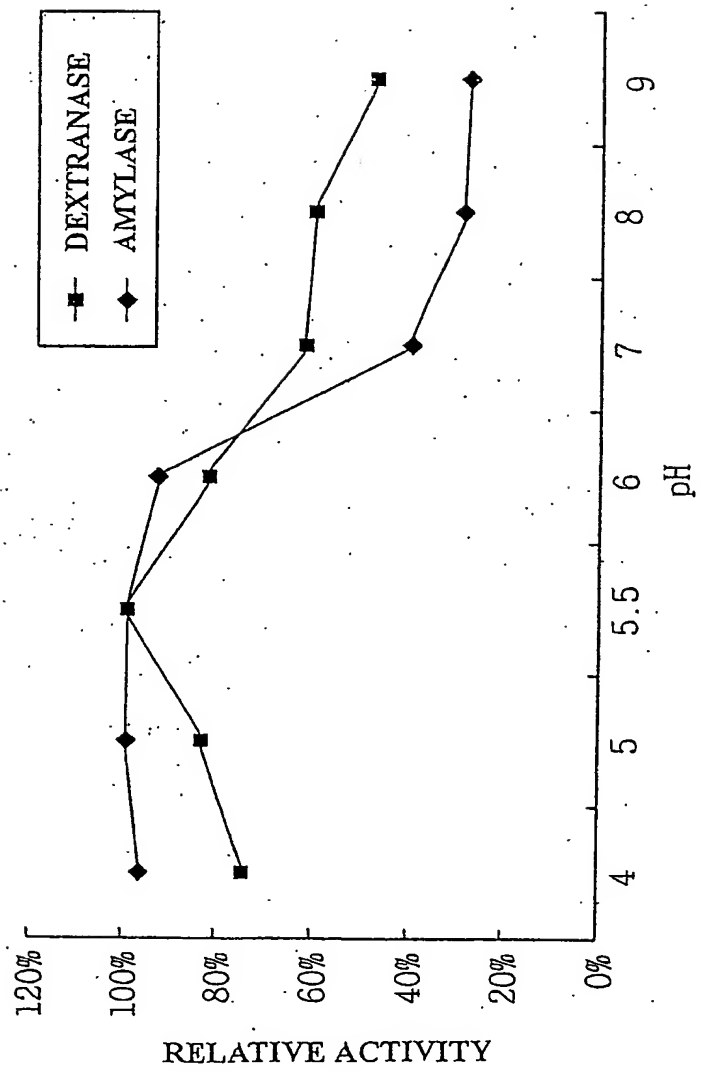
6. The cell according to claim 5, which are prokaryotes or eukaryotes.

7. The cell according to claim 6, which are *E. coli* KCTC 10024BP.

8. A process for preparing enzyme degrading insoluble glucan and having both dextranase activity and amylase activity, which comprises the steps of (a) culturing cells of claim 5; (b) expressing enzyme from the cultured cells; and (c) purifying the enzyme.

9. Enzyme prepared by the process of claim 8.

10. A composition comprising the enzyme of claim 9.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AMYLASE
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100%
80%
60%
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20%
0%
RELATIVE ACTIVITY
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5
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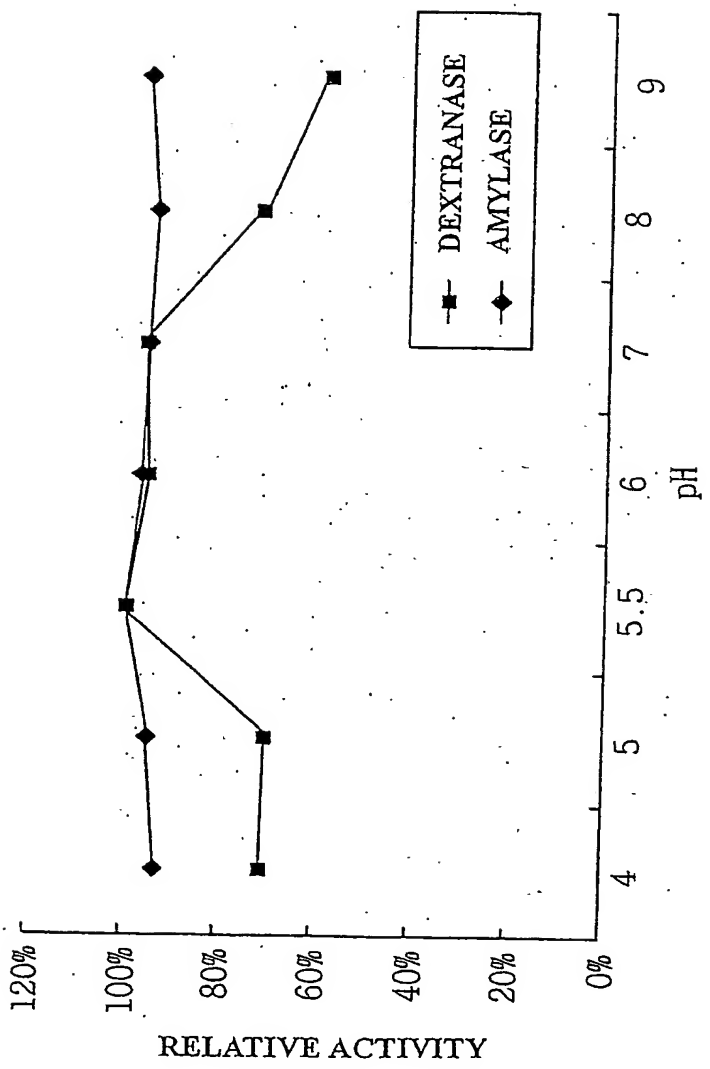
6
7
8
9
pH

FIG. 1a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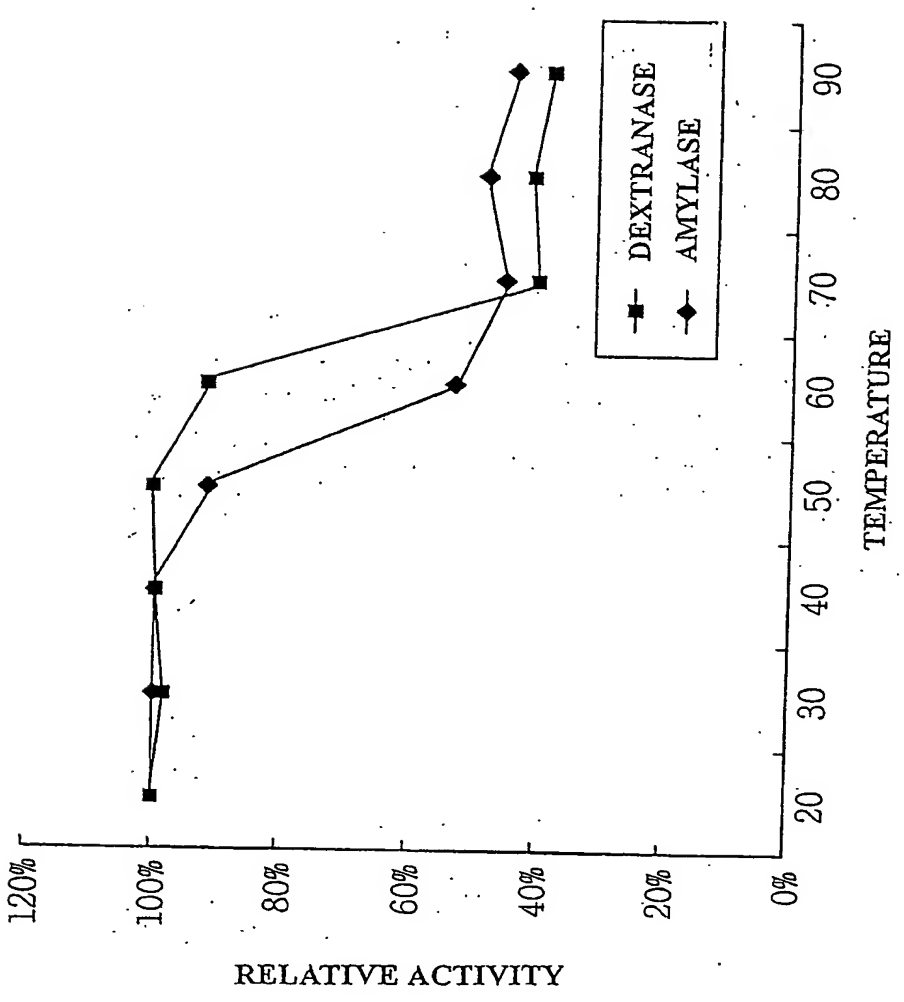
9
pH

FIG. 1b


DEXTRANASE
AMYLASE
120%
100%
80%
60%
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0%
RELATIVE ACTIVITY
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60
70
80
90
TEMPERATURE

FIG. 2

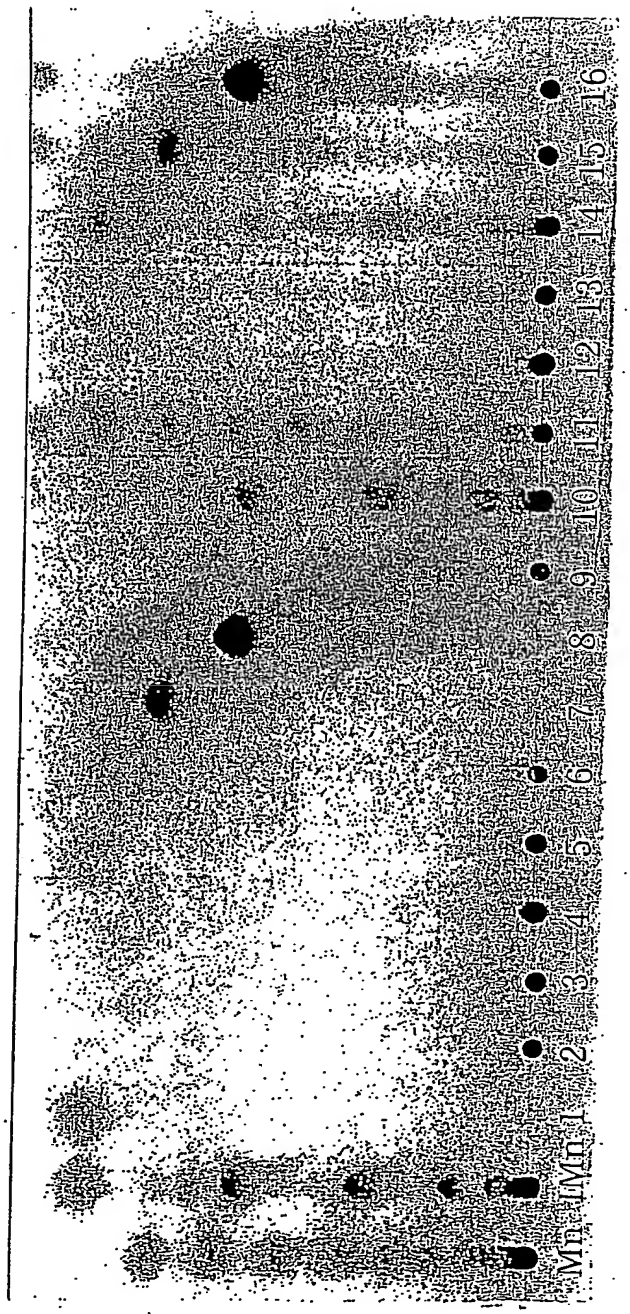
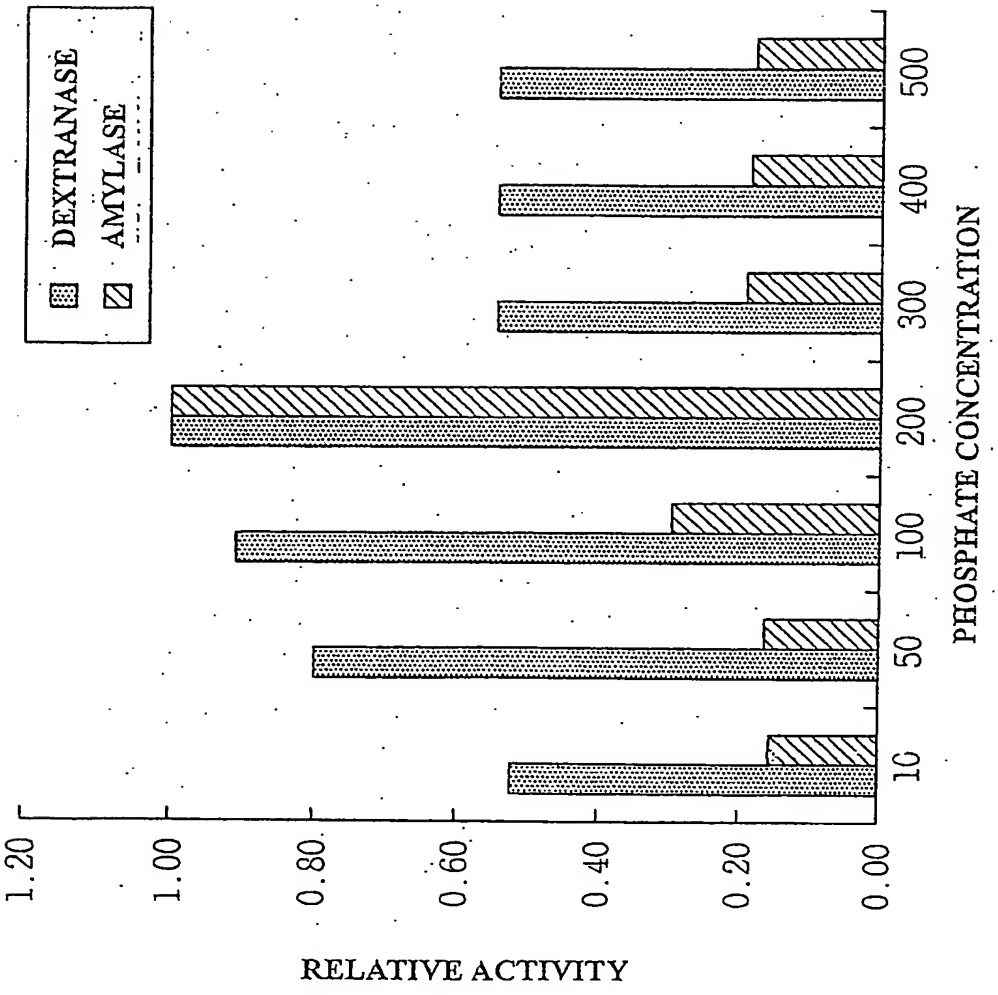


FIG. 3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AMYLASE
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1.00
0.80
0.60
0.40
0.20
0.00
RELATIVE ACTIVITY
10
50
100
200
300
400
500
PHOSPHATE CONCENTRATION

FIG. 4

Sequence Listing

<110> KIM, DO MAN
LIFENZA CO., LTD.: KIM SUNG HYUK

<120> Enzyme with the removal activities of the plaques, DNA sequence encoding said enzyme, the expressing host cell and methods for producing and purifying said enzyme

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<170> KopatentIn 1.71

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<211> 892
<212> PRT
<213> E. coli XL1-Blue/pBDEXAM

<400> 1

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Cys Ser Ile Pro Val Pro Ile Trp Ile Pro Trp Ala Leu Gln Leu Gly
35 40 45

Ile Pro Ile Leu Pro Pro Ser Leu Tyr Leu Tyr Gly Ile Ser Val Gly
50 55 60

Ile Ser Ile Arg Lys His Leu Ser Asn Gln Arg Leu Pro Cys Ala Tyr
65 70 75 80

Leu Leu Gln Val Pro Cys Ser Ser Glu Pro Ser Cys Tyr Asn Asn Arg
85 90 95

His Gly Ser Cys Pro Leu Tyr Asp Ser Ile Leu Trp Ile Gly Ser Gly
100 105 110

Arg Asn Tyr Ala Asn Leu Ser Ser Gln Leu Val His Thr Trp Leu Val
115 120 125

Sequence Listing

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Gly Leu Thr Thr Gly Ser Ser Ile Tyr Arg Gln Ile Ser Gln Tyr Ala
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Thr Ile Ile Ile Gly Leu Gln Phe Leu Ala Gln Phe Leu Leu Asn Leu
165 170 175

Leu Cys Asp Arg Cys Gly Val Val Gly Leu Ser Ser Phe Leu Gly Ser
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Ser Pro Met Arg Thr Ala Ala Met Gly Gln Leu Ser Ala Leu Ala Val
195 200 205

Pro Leu His Pro Asn Gly Asn Ser Thr Gly Cys Cys Arg Ala Val Val
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Thr Leu Phe Gln Thr Arg Asn Ser Trp Pro Thr Arg Thr Thr Gly Leu
225 230 235 240

Arg Lys Ser Leu Val Trp His Gly Lys Ser Arg Leu Tyr Thr Thr His
245 250 255

Ala Tyr Thr Pro Phe Arg Ser Tyr Ser Tyr Arg Arg Leu Glu Cys Gln
260 265 270

Val Ser Tyr Ser Thr Pro Thr Arg Cys Ile Pro Tyr Gly Ser Arg Ser
275 280 285

Leu Met Gln Leu Asp Leu Glu Val Arg Ile His Ile Arg Leu Val Leu
290 295 300

Ile Leu His Leu Pro Gly Leu Phe Ser Ser Leu Cys Val Gln Gly Ala
305 310 315 320

Leu Ala Gly Val Thr Val Lys Gln Asn Asn Ala Ala Ala His Ala Ile
325 330 335

Sequence Listing

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Ile Ala Val Ile Cys Asp Ser Pro Asn Gln Pro Met Trp Tyr Thr Ser
355 360 365

Ile Leu Thr Gly Ile Gln Ala Trp Asn Cys Gly Gly Lys Thr Ile Asn
370 375 380

Ala Gln Pro Ser Lys Thr Ile Tyr Phe Asp Ala Thr Thr Gly Leu Val
385 390 395 400

Trp Val Ala Ser Arg Val Ser Thr Arg Trp Lys Ile Trp Met His Asn
405 410 415

Leu Arg Ile Leu Thr His Arg Gln Arg Thr Leu His Pro Tyr Glu Thr
420 425 430

Gly Thr Leu Tyr Ile Ser Gly Ile Gly Asp Phe Tyr Asp Leu Thr Glu
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Thr Ser His Cys Gln Glu Ser Met Thr Pro His Ile Ala Asp Leu Asp
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Pro Ala Leu Pro Gly Ser Lys Phe Cys Ala Pro Trp Pro Gln Ser Gln
465 470 475 480

Lys Pro Asn Leu Trp Ile Arg Gly Gly Thr Tyr Leu His His Phe Val
485 490 495

Pro Ala Gly Thr Leu Ala Leu Gly His His Val Ser Val Gly Ser Thr
500 505 510

Ser Ser Asp Ala Ala Ile Phe Ser Arg Ile Val Ser Cys Trp Arg Val
515 520 525

Val Leu Ala Val Tyr Cys Val Ser Phe Ile Ala Ala Arg Ser Arg Asn
530 535 540

Ile Val Glu Tyr Cys Ala Ala Arg Gln Phe Pro Ser Met Met Met Gln

Sequence Listing

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Ile Pro Gly Lys Ile Ser Gly Val Val Met His Glu Pro Ile Gly Val
595 600 605

Ile His Thr Lys Tyr Ile Lys Asp Gly Thr Ala Phe Gly Tyr His Trp
610 615 620

Pro Leu Trp Ile Leu Cys Asn Leu Asp Pro Ile Tyr Asn Cys Ser Ser
625 630 635 640

Arg Tyr Arg Asn Val Val Cys Glu Leu Leu Cys Pro Thr Leu Phe Arg
645 650 655

Cys Ser Ser His Pro Ala Ala Ile Asp Asn Arg His Asp Val Cys Gly
660 665 670

Arg Leu Leu Ala Ser Ser Arg His Arg Phe Leu Ser Arg Val Leu Lys
675 680 685

Phe Gly Thr Ala Glu Ser Leu Thr Arg Tyr Gln Val Glu Asn Phe Ile
690 695 700

Val Lys Arg Gln Val Asp Ile Leu Arg Val Asp Leu Leu Arg Pro Arg
705 710 715 720

Pro Ser Asp Gln Arg Leu Ser Glu Ser Ala Met Ala Ala Arg Val Gly
725 730 735

Ala Ala Lys Tyr Arg Arg Arg Leu Ile Arg Ala Lys Ser Arg Leu Tyr
740 745 750

Ala Thr Gln Lys Met Ile Glu Asp Arg Glu Met Glu Ile Ala Arg Leu
755 760 765

Sequence Listing

Lys Gln Arg Ile Arg Glu Ser Arg Leu His Arg Asp Ser Thr Arg Ser
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Gly Phe Ala Arg Tyr Gly Gln Asn His Gly Ser Lys His Arg Ala Gly
785 790 795 800

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Sequence Listing

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Sequence Listing

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR01/01439

A. CLASSIFICATION OF SUBJECT MATTER

IPC7 C12N 9/24, C12N 15/56, C12N 1/20

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N 9/24, C12N 15/56, C12N 1/20

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the intertnational search (name of data base and, where practicable, search terms used)
GenBank, PubMed, CA, Espacenet, PAJ, USPTO, "Lipomyces starkeyi", "amylase", "dextranase"

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Kim, D. et al., "Characterization of a novel carbohydrase from Lipomyces starkeyi KSM 22 for dental application", J. Microbiol. Biotechnol., 9(3), 260-64, 1999.	1-10
X	Ryu, S.J. et al., "Purification and partial characterization of a novel glucanhydrolase from Lipomyces starkeyi KSM 22 and its use for inhibition of insoluble glucan formation", Biosci. Biotechnol. Biochem., 64(2), 223-8, 2000.	1-10
E	WO 01/66570 A1 (Kim, D. et al.), 13 Sep. 2001.	1-10
A	Kelly, C.T. et al., "Thermostable extracellular alpha-amylase and alpha-glucosidase of Lipomyces starkeyi", Appl. Microbiol. Biotechnol., 22(5), 352-8, 1985.	1-10
A	Koenig, D.W. et al., "Induction of Lipomyces starkeyi dextranase", Appl. Environ. Microbiol., 55(8), 2079-81, 1989.	1-10

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevence
"E" earlier application or patent but published on or after the international filing date
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"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevence; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevence; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents,such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 MAY 2002 (21.05.2002)	23 MAY 2002 (23.05.2002)
Name and mailing address of the ISA/KR Korean Intellectual Property Office Government Complex-Daejeon, 920 Dunsan-dong, Seo-gu, Daejeon Metropolitan City 302-701, Republic of Korea Facsimile No. 82-42-472-7140	Authorized officer LEE, Cheo Young Telephone No. 82-42-481-5594

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